III. Specification amendments

Paragraph beginning at line 5 on page 1:

This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Application Serial No. 60/059,252, filed on September 18, 1997 and the benefit of priority under 35 U.S.C. §120 to U.S. Patent No. 6,375,954 ~~Application Serial No. 09/157,257~~, filed on September 18, 1998.

Paragraphs beginning on page 3, line 22:

FIG. 2a: Part 1 of 3 parts of a Nucleotide sequence of 85 kD gene with flanking regions and deduced amino acid sequence of the strain-specific antigen from E. risticii 90-12 strain (SEQ ID NO: 3 and 4). Putative -10, -35, RBS regions are underlined and putative starts of transcription is denoted (+1). The dyad symmetry, and the adjacent thymine-rich regions are underlined.

FIG. 2b: Part 2 of 3 parts of the sequence of Figure 2a.

FIG. 2c: Part 3 of 3 parts of the sequence of Figure 2a.

FIG. 3a: Part 1 of 2 parts of a Nucleotide sequence of 50 kD gene with flanking regions and deduced amino acid sequence of the strain-specific antigen from E. risticii 25-D strain (SEQ ID NO: 5 and 6). Putative -10, -35, RBS regions are underlined and putative starts of transcription is denoted (+1). The dyad symmetry, and the adjacent thymine-rich regions are underlined.

FIG. 3b: Part 2 of 2 parts of the sequence of Figure 3a.

FIG. 4a: Part 1 of 2 parts of a Nucleotide sequence of ATCC-50 kD gene with flanking regions and deduced amino acid sequence (SEQ ID NO: 7 and 8). Putative -10, -35, RBS regions are underlined and putative starts of transcription is denoted (+1). The dyad symmetry, and the adjacent thymine-rich regions are underlined.

FIG. 4b: Part 2 of 2 parts of the sequence of Figure 4a.

Paragraph beginning on page 5, line 16)

The SSA may vary widely in both overall size and amino acid composition. The SSA may have a molecular weight of about 40 to about 90 kDa, inclusive of all specific values and subranges therebetween. In specific embodiments of the present invention, the SSA has a molecular weight of about 50 kDa or 85 kDa. Examples of specific amino acid sequences of the inventive SSAs are shown in FIGS. 2-4 (SEQ ID NO: ~~3, 5, and 7~~ 9, 6 and 8).

The present invention also provides isolated and purified nucleic acids (e.g., recombinant DNAs) which encode the SSAs. Specific examples of nucleotide sequences encoding the SSA of the present invention are shown in FIGS. 2-4 (SEQ ID NO: ~~4, 6, and 8~~ 3, 5 and 7). All nucleotide sequences encoding a particular SSA are included in the scope of the present invention. Selecting a nucleic acid encoding a particular amino acid sequence may be readily accomplished using the well-established genetic code relating the nucleic acid sequence of a codon sequence to the amino acid sequence encoded thereby. The genetic code is provided by R. H. Abeles et al, Biochemistry, Jones and Bartlett, 1992, p. 269, incorporated herein by reference in its entirety.

(Paragraph beginning on page 34, line 17)

The complete 50 kD and 85 kD genes were amplified separately from the genomic DNA of the original and variant strain of E. risticii by using two modified primers, named as expression

cloning primers E.C.P-1 and E.C.P-2 (FIG. 1). The E.C.P-1 (.sup.5' CAT AAA ATT TCT AAG ACG AAG GAT CCC TAT GTC.sup.3') (SEQ ID NO. 9) was selected from the known sequence of bp upstream of the first methionine codon of the genes. This 33 base primer was modified at base 21 and 22 position by the substitution of two A's in it original sequence with two G's. In the same way, E.C.P-2 (.sup.5' GAG AGA AAG TTC CCC GTG TGA ATT CTA GCT AGG.sup.3') (SEQ ID NO. 10) was selected from the known sequence 69 bp downstream of the stop codon of the gene. This 33 base primer also was modified at base 21 by introducing another single base A. Amplification of the complete genes (50 kD and 85 kD) by using these two modified primers produced BamH I and EcoR I sites at the extreme 5' and 3' end of the genes respectively.

(paragraph beginning on page 42, line 6)

EcoR I restriction digestion of the 50 kD .lambda.-gt11 recombinant phage DNA, generated a 3.9 kb insert DNA fragment which was cloned in pBluscript SK(+) phagemid for restriction mapping. Fifteen restriction enzymes (6 base-cutters) were used to determine the presence of restriction sites in the insert DNA of the above pBluscript SK(+) subclone. The Hind III digestion of the recombinant pBluscript SK(+) DNA produced three DNA fragments of 3.5 kb, 2.2 kb and 1135 bp. The 3.5 kb DNA fragment was a plasmid-insert DNA piece, where 565 bp was an insert part and the rest of it was pBluscript SK(+) phagemid. This specific fragment was re-circularized to form a pB50-6.1 subclone. The 2.2 kb and 1135 bp insert fragments were subcloned separately in pBluscript SK(-) phagemid and they were designated as pB50-6.2 and pB50-6.3 respectively. It was difficult to select a primer for downstream sequencing of the pB50-6.2 recombinant clone, due to the presence of direct repeats in the middle of the insert. In order to overcome this situation an internal segment of 826 bp was PCR amplified by using two unique primers: 50-A (.sup.5' ATA

CTA AAA AGC ATA CTC.sup.3') (SEQ ID NO. 11) and 50-B (.sup.5' TTC TAC AAG CCC TTT AAA.sup.3') (SEQ ID NO. 12). The amplified product was cloned in pCR.TM. vector and designated as pCR50-6.2.1. The insert piece of the pCR50-6.2.1 recombinant clone was then easily sequenced by using the universal primers of the vector. The presence of direct repeat motifs in the pB50-6.3 recombinant clone produced the same problem as described above and thus the insert piece of this clone was further subcloned in smaller fragments to exploit the advantage of the universal primer sequences for the vector. For this purpose the restriction digestion was performed with Pst I and the generated fragments were cloned separately in pBluscript SK(-) phagemids. Subclones were designated as pB50-6.3.1 and pB50-6.3.2.

The two in vivo excised phagemid clones partially expressing the 85 kD antigen gene were designated as pB85-11 and pB85-17. The insert size of these two clones were 4.5 kB and 1.1 kb respectively. These two clones had 58% overlapping regions with each other and they together covered 84% of the 85 kD gene sequence. The remaining unknown 16% of the 5' region of the gene was separately cloned by PCR from 90-12 genomic DNA, using primers 50-C (.sup.5' GAA TGT TCA GCT TTC CGG.sup.3') (SEQ ID NO. 13) and 50-D (.sup.5' AGC TGT ATC GTT CGT GAG.sup.3') (SEQ ID NO. 14) The 1.5 kb amplified product was cloned in pCR.TM.II vector and designated as pCR85-3. The 3' region of the gene was covered by the pB85-11 recombinant clone. The presence of too many direct repeats in this region made the selection of sequencing primers extremely difficult. To overcome this situation the insert segment of this clone was further subcloned in smaller fragments to exploit the advantage of the universal primer sequences for the vector. For this purpose two primers, 85-E (.sup.5' GTA TAC TTA CAG ATA GCA C.sup.3') (SEQ ID NO. 15) and 50-E (.sup.5' GCC GAC AGT ATC ATT AAA C.sup.3') (SEQ ID NO. 16),

were used to amplify a 876 bp segment, using pB85-11 recombinant DNA as a template. The segment was cloned separately in a pCR.TM.II vector and designated as pCR85-11.1. The insert piece of pCR85-11.1 was restriction digested with Hind III enzyme and as a result of this, two DNA fragments of 4.3 Kb and 443 bp were produced. The 4.3 Kb fragment consisted of 495 bp insert piece and the rest of it (3.8 kb) was the plasmid vector part. This specific fragment was re-circularized to form the pCR85-11.1.1 subclone. The 441 bp fragment consisted of a 383 bp insert piece and a 60 bp plasmid piece. The 441 bp fragment was subcloned at the Hind III site of the pBluscript SK(-) phagemid and designated as pB85-11.1.2. The recombinant DNA of pCR85-11.1.1 was double digested with Hind III and EcoR I. The generated fragments were purified from the agar gel by the Gene clean technique and were further restriction digested with Sau3A I enzyme. The Sau3A I digestion generated two fragments of 317 bp and 247 bp. These fragments had a 9 bp and a 60 bp of plasmid sequence, respectively. These two pieces were separately subcloned at BamH I-EcoR I and BamH I -Hind III sites of pBluscript SK(-) phagemid. They were designated as pB85-11.1.1.1 and pB85-11.1.1.2 respectively.

(On page 47, line 20, please replace the table with the following:

Type of Repeats	Repeat Sequence	Repeated from Base
I	(SEQ ID NO. 17) AAAGAAATACT	957, 1434, 777, 1287, 1353, 648.
II	(SEQ ID NO. 18) CAAATACTCAC	807, 1356, 651, 1290, 1383.
III	(SEQ ID NO. 19) AAATTTAAAGA	978, 1242, 852, 1110, 915.
IV	(SEQ ID NO. 20) CTAAACACAT	510, 1017, 891, 1149.
V	(SEQ ID NO. 21) AAAGACATACT	501, 1071
VI	(SEQ ID NO. 22) TTTAAAGAGCT	342, 1113.
VII	(SEQ ID NO. 23) ATTTTTTATAA	75, 119.
VIII	(SEQ ID NO. 24) AACTTTAAAGG	408, 1179.

IX	(SEQ ID NO. 25) AAGTTTAAACA	339, 1584.
X	(SEQ ID NO. 26) TACTCACTAAT	457, 1504.
XI	(SEQ ID NO. 27) ACTTTAAAAAA	669, 1309.
XII	(SEQ ID NO. 28) ATAAGTTTAAA	237, 288

(On page 49, line 1, please replace the table with)

Type of Repeats	Repeat Sequence	Repeated From Base.
I	(SEQ ID NO. 29) ATACTTACAGA	652, 1963, 1300, 901, 832, 385, 2260, 1729, 316.
II	(SEQ ID NO. 30) AAATTTAAAGA	1984, 2116, 1852, 1390, 1252, 853, 784, 1915.
III	(SEQ ID NO. 31) CTAAAAGAGAT	1891, 2023, 1429, 892, 760, 376, 1228.
IV	(SEQ ID NO. 32) AAAGAAATACT	1696, 1567, 1165, 2227, 1030, 2161, 646.
V	(SEQ ID NO. 33) TACTTACAGAT	1064, 1964, 834, 902, 1301, 1730, 2261.
VI	(SEQ ID NO. 34) AAAGACATACT	310, 1945, 1351, 1282, 883, 814, 367.
VII	(SEQ ID NO. 35) ACAGCTAAAGA	2275, 2302, 1771, 1744, 2302, 1159.
VIII	(SEQ ID NO. 36) TTTAAAGAACT	1393, 2515, 2185, 856, 1323, 339.
IX	(SEQ ID NO. 37) GAAATACTTAC	2164, 2257, 641, 1168, 1726.
X	(SEQ ID NO. 38) AGCACTGGTAA	1975, 2005, 1381, 844, 1312.
XI	(SEQ ID NO. 39) GATAAATTTAA	1912, 2380, 781, 1249, 1849.
XII	(SEQ ID NO. 40) CTTATAGAAAG	934, 1333, 865, 349, 550.
XII	(SEQ ID NO. 41) GAAATACTCAC	676, 2230, 1699, 1570, 1033.
XIV	(SEQ ID NO. 42) ACCGGTAACTT	532, 916, 2230, 1699, 1570, 1033.
XV	(SEQ ID NO. 43) ATGCAACAAAA	2204, 2621, 1007.
XVI	(SEQ ID NO. 44) GCTAAAGAAGT	1189, 2278, 1747.
XVII	(SEQ ID NO. 45) CTTACAGATAA	904, 2035, 1441.
XVIII	(SEQ ID NO. 46) GCAATAACTGG	733, 1864.
XIX	(SEQ ID NO. 47) ATGGTAAGGAC	494, 746.
XX	(SEQ ID NO. 48)	417, 1401.

ACTTATAGAAG